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Investigation 12: Fruit Fly Behavior

Background

Drosophila melanogaster, the common fruit fly, is an organism that has been studied in the scientific community for more than a century. Thomas Hunt Morgan began using *Drosophila melanogaster* for genetic studies in 1907. The common fruit fly lives throughout the world and feeds on the fungi of rotting fruit. It is a small fly, and one could question why so much time and effort have been directed to this organism. It is about the size of President Roosevelt's nose on a dime, but despite its small size, the fly is packed with interesting physical and behavioral characteristics. Its genome has been sequenced, its physical characteristics have been charted and mutated, its meiotic processes and development have been investigated, and its behavior has been the source of many experiments. Because of its scientific usefulness, *Drosophila* is a model research organism. Its name is based on observations about the fly; the fly follows circadian rhythms that include sleeping during the dark and emerging as an adult from a pupa in the early morning. This latter behavior gave rise to the *Drosophila* genus name, which means "lover of dew." The explanation for the species name *melanogaster* should be clear after observing the fly's physical features. It has a black "stomach," or abdomen. No doubt the dew-loving, black-bellied fly will continue to make contributions to the scientific community and to student projects.

This investigation explores the environmental choices that fruit flies make. A choice chamber is designed to give fruit flies two choices during any one test. A *taxis* (from Greek *taxis* meaning to arrange) is an automatic, oriented movement towards or away from a stimulus. Movement toward a substance is a positive taxis. Consistent movement or orientation away from a substance is a negative taxis. Animals move in response to a variety of stimuli. A *chemotaxis* is the movement in response to a chemical stimulus. *Phototaxis* is movement in response to light. *Geotaxis* is movement in response to gravity. The flies could also exhibit a behavior that is not oriented toward or away from the stimulus; rather, the stimulus elicits a random response. Such behavior would be considered a kinesis. In most cases, the experiments done in the choice chamber will be chemotactic experiments, as indicated by the number of flies that collect on one end of the chamber or another in response to a chemical stimulus. Students will also investigate if the chemotactic response is greater than a geotactic or phototactic response.

Because flies are very common in households (in fact, fruit flies live almost everywhere that humans live), think about using food or condiments that might result in a positive or a negative chemotactic response from the flies. Think about how an organism benefits by responding to chemicals differently in their environment. As the old saying goes, "You attract more flies with honey than with vinegar!" Let's see if this is true…

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Pre-Lab Questions

1. Classify each of the following examples with regards to the direction AND type of taxis (ie- positive or negative AND photo, geo, or chemotaxis)

- Infant rats placed on a shallow incline oriented and moved downhill within 1 minute
  -
- Planaria (flatworms) avoid light
  -
- Stink bugs release chemicals that attract other stinkbugs
  -

2. List 10 household substances (foods, condiments, chemicals, etc.) you would be interested in using to investigate fruit fly responses

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-
-
-
-
-
-
-
-
-

3. Which of those substances do you think would attract vs repel flies? Why?

4. How do you think the fruit flies will respond to light vs gravity? Justify your answer.

5. How can you alter the chamber to investigate each of those variables (ie-light and gravity)?

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Procedure

Handling Drosophila Cultures and Tossing Flies

1. Using established *Drosophila melanogaster* cultures, carefully toss between 10 –30 living flies into an empty vial. Be sure to plug the vial as soon as you add the flies. Do not anesthetize the flies before this or any of the behavior experiments

- Tossing Flies: When flies are "tossed" they are tapped into an empty vial. It might be a good idea to take the existing vial and place it in the refrigerator for a minute or two to slow the movement of the flies –Drosophila are ectotherms, so their metabolic rates are directly linked to the ambient temperature. Tap a culture vial (push the vial down on a solid surface several times) on the table to move the flies to the bottom of the vial. Quickly remove the foam or cotton top and invert an empty vial over the top of the culture vial. (Note: A funnel may be placed in the receiving vial to reduce chances of the flies escaping.) Invert the vials so that the culture vial is on the top and the empty vial is on the bottom, and tap the flies into the empty container by tapping it on a solid surface several times. Be sure to hold the vials tightly to keep them together. You must then separate the vials and cap each separately. Do not try to isolate every fly from the original culture. It is difficult to separate flies, and you may lose a fly or two in the process.

Preparing the "Choice Chamber"

2. Using a ruler, determine the distance half-way across the transparent plastic cylinder, approximately 6" from the end.
3. Using a permanent marker, draw a vertical line at this half-way point.
4. Label one side of the chamber "A" and the other side "B."
5. Take two cotton balls and place 5 drops of water on each. Do NOT saturate the cotton – if liquid starts to run down the side of the choice chamber, it will trap the flies.
6. Insert a dissection pin through the center of the cotton ball.
7. Attach the cotton ball to the foam plug.

Control Run

8. Place one of the foam plugs with its attached water cotton ball into one end of the choice chamber.
9. Attach a funnel to the other side of the choice chamber. Carefully, take your vial of about 10–30 flies and toss them into the chamber. The flies should "fall" to the cotton plug at the other side of the choice chamber.
10. Quickly remove the funnel and fit the other foam plug with its attached water cotton ball into the other side of the choice chamber. This will be our control group run.

- Since there is no substance except for water on either side of the chamber, there should be no preference exhibited by the fruit flies. They should be relatively equally distributed in both regions.

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11. Lay this control group down on a white surface (for easier observation) on a flat (think about why!) surface. It should be easy to see the labels on the choice chamber along with the distribution of flies.
12. Give the flies 5 minutes of undisturbed time, and then count (it would be a good idea to take a picture with your phone to "capture the moment") the number of flies at each section of the chamber. Record your observations in Table 1.
13. To increase your sample size, record the data from each group and then total the class results.
14. Using the class totals, carry out a chi-square analysis to accept or reject the NULL HYPOTHESIS that there is no preference. (This is good chi-square practice and also you need to "show" that there is no preference!)

Baseline Activity

15. Now hold the choice chamber vertically. Record which direction the fruit flies are traveling (A).
16. Turn the chamber over and wait 30 seconds. Observe the flies again and note their direction of travel (B).
17. Return the chamber to a horizontal position and wait 30 seconds. Shine light on one end of the chamber. Once again, observe the flies and record their direction of travel (C).
18. Follow directions given to "dispose" of the flies. They might be used again tomorrow, or they might wind up in the morgue...
19. Complete the analysis questions for the baseline activity.

Results

Control Run

Table 1. Fruit Fly Choices: Water vs Water (Control)

Side of Choice Chamber	Number of Flies						
	Red	Orange	Yellow	Green	Blue	Purple	TOTAL
A (Water)							
B (Water)							

Baseline Activity

Observations:

A. When chamber was oriented vertically:

B. When chamber was turned over:

C. When light was shone on one end of horizontal chamber:

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Analysis

Control Run

- Write your null hypothesis
  -
  -
- Formalize your thinking by making a contingency box

- Calculate your chi-square value
  - Show your work:

$$\chi^2 = \sum \frac{(\text{Observed Value} - \text{Expected Value})^2}{(\text{Expected Value})}$$

  - Chi-square value =

- Determine the critical chi-square value (use a p-value of 0.05)

p value	Degrees of Freedom							
	1	2	3	4	5	6	7	8
0.05	3.84	5.99	7.82	9.49	11.07	12.59	14.07	15.51
0.01	6.64	9.21	11.34	13.28	15.09	16.81	48.48	20.09

  - Degrees of freedom =
  - Critical chi-square value =

- Draw your conclusion
  - Our calculated chi-square value is _____ than our critical chi-square value
  - Therefore, we _____ our null hypothesis which means
    -
    -

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Baseline Activity

- To what stimulus were the flies responding in part
  - A/B:
  - C:
- **Identify** the direction AND type of taxis exhibited by the fruit flies in parts A/B **AND explain** your reasoning
- **Identify** the direction AND type of taxis exhibited by the fruit flies in part C **AND explain** your reasoning
- How did you try to make sure the fruit flies were only responding to one stimulus at a time?
- How could you determine if one taxis is stronger than another taxis?

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Inquiry-Based Formal Lab: Testing Different Chemical Stimuli

You will design an investigation to determine commonalities in substances that attract and repel fruit flies, as well as their reaction to light and gravity. Use the procedures and information learned in this lab so far, as well as the list compiled as a class from the pre-lab suggestions, to guide your design to test the reactions of fruit flies to different household substances. Your group will be responsible for bringing in these items for the investigation.

- Note: Substances may be liquid, following the cotton ball method used in the control run. Solid pieces of fruit or other food may also be used by attaching a sample directly to the foam plug with a dissection pin.

You must prepare **<u>at least 6</u>** different choice chamber set-ups to investigate the following:

- 3 chemotactic responses
  - Variable A vs control
  - Variable B vs control
  - Variable C vs control
- 1 chemotactic vs chemotactic response
  - Chemical variable vs Chemical variable
- 1 chemotactic vs geotactic response
  - Chemical variable vs gravity
- 1 chemotactic vs phototactic response
  - Chemical variable vs light

As you plan, discuss, evaluate, execute, and justify an experiment to test a question regarding taxis and *Drosophila*:

- Decide upon one question your group would like to explore at a time
- Develop a testable hypothesis
  - Note: Your hypothesis about substance preference is NOT the same as the NULL HYPOTHESIS that you will use in the chi-square analysis…make sure you understand this! Rather, this is your alternate hypothesis.
- Discuss and design a controlled experiment to test the hypothesis using the behavior chamber
  - Hint: Large sample size would help…Figure out how to do this. (There is more than one way!)
- List all materials required and be aware of safety precautions
- Determine how to collect and record data
- Determine how the data will be analyzed to test the hypothesis
- Review your hypothesis, safety precautions, procedure, and proposed data analysis with your instructor to be approved prior to beginning the experiment
- Once the experiment and analysis are complete, evaluate your hypothesis and justify whether or not the hypothesis was supported by your data
- Identify potential sources of error and make suggestions for a revised or new experiment to modify or retest your hypothesis

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Format for Formal Lab Write-Up

You will individually complete a formal lab report on the inquiry portion of this behavior lab using the following template. The final lab report must be typed (including tables and graphs, although calculations can be hand-written) and should be proofread for spelling and grammatical errors. Each section should consistently be written in the first-person active voice. (Ex: We counted the flies.)

Title:

The title states the focus of your experiment. The title should be to the point, descriptive, accurate, and concise (ten words or less). Do not make it too general!

Introduction:

The introduction of a lab report should include the following elements:

- State the purpose of your experiment
  - Why are you doing this experiment? What question(s) are you trying to answer?
  - Include important preliminary observations or background information about the subject
    - Include key vocabulary terms and explanations
    - Include relevant information learned in the baseline/control activities
    - Note: Do NOT just copy down the background given to you in the lab packet
- Your hypothesis
  - Include both null and alternate(s)
  - Make sure the statement is testable

To be sure that you have a good understanding of your experiment, you may want to revisit the introduction after you have completed the materials and procedure, results, and conclusion sections of your lab report.

Materials and Procedure:

This section of your lab report involves producing a written description of the materials used and the methods involved in performing your experiment.

- Make a list of ALL items used in the lab
- Write a paragraph (complete sentences) which explains what you did in the lab as a short summary
- Then write a detailed step-by-step procedure in such a way that anyone else could repeat the experiment
  - Indicate when and how the materials from your list were used during the process of completing your experiment

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Results *(Data, Graphs, Calculations)***:**

This section should include any observations, data tables, graphs, calculations, or additional notes you make during the lab.

- All of your data needs to be organized into tables
- All of your data needs to then be visually displayed as graphs
    - You may have a separate graph for each data table or combine related data into the same graph
    - Note: Bar graphs would be most appropriate for the data in this lab
- Show all of your work for your calculations (ie-chi square analysis)
- All tables and graphs should be labeled appropriately.
    - This includes titles, units, headings, axis, scales, keys, etc.
- For EACH of your tables, graphs, and calculations, include a written summary (one or two lines) of the information shown. Describe any patterns or trends observed. Do NOT just restate the numbers. Do NOT interpret or explain data here.

Discussion and Conclusion:

This section is where you summarize what happened in your experiment. You will want to fully discuss and interpret the information.

- Did you accept or reject your hypothesis?
    - EXPLAIN WHY you accepted or rejected your hypothesis using data from the lab
    - What does this mean?
- Include a summary of the data - averages, highest, lowest, etc. to help the reader understand your results (Do not just copy your data here. You should summarize and reference KEY information. What trends do you notice?)
- Explain what you learned and describe how it applies to a real-life situation.
- Discuss possible errors that could have occurred in the collection of the data (experimental errors)
    - Provide suggestions regarding how your experiment could be improved upon to reduce those errors

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Consider the following questions as you design your experiment and evaluate your results. They can be incorporated into your discussion and conclusion.

- Are all substances equally attractive or repellant to the fruit flies?
- Which substances do fruit flies prefer the most?
- Which substances do fruit flies prefer the least?
- Do preferred substances have any characteristics in common?
- What other factors might affect whether or not the fruit flies moved from one part of your choice chamber to another?
- Do you think that it is the fruit itself that attracts the flies? Should they be called *fruit flies* or something else?
- What factors must be controlled in an experiment about environmental variables and behavior?
- What is the difference among phototaxis, chemotaxis, and geotaxis? Do fruit flies demonstrate all of them?
- Does a phototactic response override a chemotactic response? What about a geotactic reponse?
- Is there anything that was shared by all of the environmental factors to which the flies were attracted?
- Is there anything that was shared by all of the environmental factors to which the flies were repelled?
- How do you explain the behavior of fruit flies in someone's kitchen or in nature based on the information you collected? Does your data explain all fruit fly movements? Explain.